New claim 48 was added as follows:

48(New). The polynucleotide of Claim 12 wherein the first cistron contains an HIV *gag* gene or portion thereof which encodes a *gag* immunogenic epitope, the second cistron encodes a cytokine, and the third cistron encodes a T-cell costimulatory element, wherein the first, second and third cistron may be presented in any combination.

49(New). The polynucleotide of Claim 48 wherein the second cistron encodes an interleukin, an interferon, or GM-CSF, and the third cistron encodes a B7 protein.

REMARKS

A Petition to extend the time for responding to the outstanding Office Action, for three (3) months, under 37 C.F.R. §1.136(a), is enclosed with this paper.

Claims 1-22, 25, 35, 39-42, 44 and 45 are pending in this application.

Applicants are pleased to see that claims 18 and 39-41 are allowed, and that claims 4-11 and 16-17 are allowable if rewritten as suggested by the Examiner.

Claims 2, 3, 4, and 42 have been cancelled, without prejudice. Applicants respectfully reserve the right to pursue the subject matter cancelled herein in a future continuing application.

Claims 1, 5, 12, 19, 20, 25, 35, 44 and 45 have been amended to more particularly point out and distinctly claim the subject matter of Applicants invention.

New claims 46-49 have been added to more particularly point out and distinctly claim the present invention.

More specifically, claim 1 has been amended to incorporate the limitation of claim 4, thus allowing for cancellation of claims 2, 3 and 4.

Claim 5 has been amended to depend from claim 1 instead of cancelled claim 2, as well as reciting proper Markush group language.

Claim 12 has been amended to recite an HIV polynucleotide wherein the first, second and third cistron may be presented in any combinational order.

Claims 19 and 20 have been amended to incorporate appropriate Markush group language.

Claim 25 has been amended to recite a mammalian host, not a vertebrate host, so as to recite a clear and positive antecedent basis from amended claim 1.

Claim 35 has been amended to more clearly state the positioning of a transcription termination sequence 3' to the second, or optionally third, open reading frame.

Claim 44 has been amended to more clearly point out and distinctly claim this portion of Applicants invention.

Claim 45 has been amended to recite "a" polynucleotide instead of "the" polynucleotide.

New claim 46 mirrors original claim 24, which was inadvertently cancelled by the undersigned attorney. New claim 46 recites proper Markush group language, which was at issue earlier in prosecution with original claim 24.

New claims 47, 48 and 49 are added to recite *gag* antigens which may be utilized in a REV-independent fashion. Support for new claims 47-49 can be found throughout the specification, such as Example 4.

No new matter is added by amendment of claims 1, 5, 12, 19, 20, 25, 35, 44 and 45 or entry of new claims 46-49.

Applicants have taken this opportunity to correct several editorial oversights concerning the continuing data for this application, which should now read as follows:

> This application is a continuation of U.S. application serial no. 08/702,502, which is the §371 U.S. national phase prosecution of PCT international application serial no. PCT/US95/02633, filed March 3, 1995, now abandoned, which is a continuation-in-part of U.S. application serial no. 207,526, filed March 7, 1994.

Double Patenting Rejection

Claims 1-3, 14-15, 42 and 44 stand rejected under the judicially created doctrine of obviousness-type double patenting under the following:

1. claims 1-4 of U.S. Patent No. 5,866,553 in view of Almond et al ((WO93/11250) or Almond et al. (GB 2 262 099A);

2. claims 1-4, 8, 10-11, 13-14 of U.S. Patent No. 5,736,524 in view of Almond et al ((WO93/11250) or Almond et al. (GB 2 262 099A).

Applicants respectfully overcome this portion of the rejection as it relates to claims 1-3 and 14-15 by amendment of claim 1 to recite the limitation of claim 4, namely a polynucleotide wherein the first of two, or optionally three, cistrons encodes at least one immunogenic epitope of a *human immunodeficiency virus*. The '553 patent discloses HPV-based constructs while the '524 patent discloses Tb-based constructs. Neither document, in combination with either Almond reference, would motivate the artisan to combine these teachings, or would suggest or teach the invention as presently recited in amended claim 1.

Claim 42 has been cancelled, thus rendering moot this portion of the rejection.

Claim 44 has been amended to delete reference to the genes expressed from both HPV and tuberculosis.

As noted above, Applicants respectfully reserve the right to pursue this cancelled subject matter in a future continuing application. However, in view of the amendment to claims 1 and 44, Applicants respectfully take the position that this double patenting rejection is overcome, and claims 1 and 44, as amended, are in proper form for allowance.

Rejection of Claims 1, 14-14 and 25 Under 35 U.S.C. §103(a)

Claims 1, 14-15 and 25 stand rejected under 35 U.S.C. §103(a) as allegedly "being unpatentable over Wolff et al (U.S. Patent No. 6,228,844) in view of Almond et al ((WO93/11250) or Almond et al. (GB 2 262 099A). Applicants respectfully overcome the present rejection by amending claim 1 to incorporate the limitations of original claim 4, namely a polynucleotide wherein the first of two, or optionally three, cistrons encodes at least one immunogenic epitope of a *human immunodeficiency virus*. Wolff et al., alone or in combination with either Almond reference, does not provide the motivation for the bi- and tri-cistronic polynucleotide vaccines as presently recited in amended claims 1, 14-15 and 25. As noted by the Examiner, Wolff does not disclose any such constructs, while the "biscistronic" constructs of Almond provide questionable teaching, at best. Almond discloses in Example 1 of the PCT publication the *in vitro* transfection of HELA cells with a plasmid construct encoding a poliovirus genome and a reporter gene, chloramphenicol acetyl-transferase (CAT). In Example 2, Almond purports to replace the CAT gene with an a DNA fragment encoding a rotovirus VP8 peptide. However, Almond cannot provide data showing expression of VP8, only that the DNA fragment still resides within the plasmid construct after *in vitro* passage through HELA cells. Therefore, Almond shows expression of a reporter gene *in vitro*, but not a more realistic antigen for use in a vaccination context. The inability of Almond to show expression of the gene of interest within an *in vitro* environment would not teach, suggest or motivate the artisan of ordinary skill to use Wolff in combination with Almond to provide for the invention as presently claimed: namely an invention based on the *in vivo* delivery and expression of the antigen of interest.

Rejection of Claims 12-13, 19-22, 25, 35, 42, 44-45
Under 35 U.S.C. §112, First Paragraph

Claims 12-13, 19-22, 25, 35, 42, 44-45 stand rejected under §112, first paragraph. Applicants respectfully overcome the claim by claim basis of this rejection as follows:

Claim 12 - claim 12 has been amended to recite an HIV polynucleotide wherein the first, second and third cistron may be presented in any combination (e.g., such as 1-2-3, 1-3-2, 2-1-3, etc.).

Claim 19 - claim 19 has been amended, as suggested by the Examiner, to recite more appropriate Markush group language, namely the recitation of various HIV coding regions.

Claim 20 - claim 20 has been amended, as suggested by the Examiner, to recite appropriate Markush group language.

Claim 25 - claim 25 has been amended to recite a mammalian host, thus having a clear and positive antecedent basis from amended claim 1.

Claim 35 - claim 35 has been amended to more precisely recite in step f) the presence of a transcription termination sequence downstream of the most 3' open reading frame.

Claim 42 - claim 42 has been cancelled.

Claim 44 - claim 44 has been amended to delete reference to the genes expressed from both HPV and tuberculosis, as well as additional amendments to specifically respond to the Examiners concerns regarding steps 8, 9, 12, 13, 14, 17, 18 and 20 of claim 44. These amendments are self explanatory and more particularly point out and distinctly claim this portion of Applicants invention.

Claim 45 - claim 45 is amended to recite "a" polynucleotide instead of "the" polynucleotide.

Applicants respectfully take the position that this rejection is overcome by amendment to claims 12, 19, 20, 25, 35 and 42, as well as cancellation of claim 42. Reconsideration and withdrawal of this rejection is respectfully requested.

In view of the amendments and comments herein, Applicants respectfully take the position that claims 1, 5-22, 25, 35, 39-41 and 44-49 are in proper form for allowance. The Examiner is invited to contact the undersigned attorney if clarification is required on any aspect of this response, or if any of the claims are considered to require further amendment to be placed in condition for allowance after entry of this Amendment.

Respectfully submitted,

Date: April 12, 2002

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MARKED-UP VERSION OF APPLICATION AS AMENDED HEREIN

IN THE SPECIFICATION:

At page 1, lines 6-7, the continuing data is amended as follows:

-- This application is a continuation of U.S. application serial no. 08/702,502, [filed March 3, 1997, now abandoned,] which is the §371 U.S. national phase prosecution of PCT international application serial no. PCT/US95/02633, filed March 3, 1995, now abandoned, which is a continuation-in-part of U.S. application serial no. [207,525] 207,526, filed March 7, 1994, now abandoned. --.

IN THE CLAIMS:

Claims 1, 5, 12, 19, 20, 25, 35, 44 and 45 were amended as follows:

1(Three Times Amended). A polynucleotide which, upon *in vivo* introduction into a mammalian cell, is non-replicating and induces the co-expression in the cell of at least two gene products, comprising:

a) a first transcriptional promoter which operates in eukaryotic cells upstream from, and in transcriptional control of, a first cistron, wherein the first cistron encodes at least one immunogenic epitope of a human immunodeficiency virus antigen;

b) a second cistron downstream from the first cistron, under transcriptional control either of the first transcriptional promoter or under control of a second transcriptional promoter;

c) optionally, a third cistron downstream from the second cistron, under transcriptional control either of the first transcriptional promoter or under control of the second transcriptional promoter, or under control of a third transcriptional promoter; and

d) a transcriptional terminator following each of the first, second and third cistron, unless said first cistron or second cistron is followed by a second cistron or third cistron, respectively, which lacks its own transcriptional promoter.

5(Amended). The polynucleotide of Claim [2] 1 wherein the first cistron encodes a human immunodeficiency virus (HIV) gene selected from the group consisting of env, gag, gag/pol, gag/protease, gag and portions of pol not encoding a functional polymerase, and pol.

12(Amended). The polynucleotide of Claim 1 wherein the first cistron encodes a REV-independent human immunodeficiency (HIV) epitope, the second cistron encodes a cytokine, and the third cistron encodes a T-cell costimulatory element, wherein the first, second and third cistron may be presented in any combination [each of the cistrons may also be presented in a different order].

19(Amended). The polynucleotide of Claim 18 wherein the HIV immunogenic epitope is selected from the group of HIV genes consisting of gag, gag-protease, and [or] env or an immunogenic subportion thereof; the cytokine is interleukin-12, and the T-cell costimulatory element is a B7 protein.

20(Amended). The polynucleotide of Claim 19 wherein the env immunogenic epitope is selected from the group consisting of HIV gp160, HIV gp120 and HIV gp41.

25(Amended). A method for co-expression in a single cell *in vivo*, of at least two gene products, which comprises introducing between about 1 ng and about 100 mg of the polynucleotide of Claim 1 into the tissue of the [vertebrate] mammal.

35(Three Times Amended). A polynucleotide which is non-replicating in eukaryotic cells *in vivo*, comprising:

a) a eukaryotic transcriptional promoter;

b) an open reading frame 3' to the transcriptional promoter encoding an immunogenic HIV epitope wherein the open reading frame has a splice donor sequence at the 5'-side of the open reading frame, a REV responsive element anywhere within the open reading frame, and a stop codon encoding the termination of translation of the open reading frame;

c) an internal ribosome entry site (IRES) 3' to the translation stop codon of the open reading frame;

d) an open reading frame encoding a spliced HIV REV gene at the 3' end of which is a translation stop codon;

e) optionally, 3' to the REV translation stop codon, a second IRES, followed by an open reading frame encoding immunomodulatory or immunostimulatory genes being selected from the group consisting of GM-CSF, IL-12, interferon, and a B7 protein; and,

f) a transcription-termination signal 3' of the most downstream open reading frame of step d) or optionally, step f) [following the last open reading frames].

~~44~~(Twice Amended). A polynucleotide which, upon *in vivo* introduction into a mammalian cell, is non-replicating and induces the co-expression in the cell of at least two gene products, the polynucleotide comprising a first transcriptional promoter which operates in eukaryotic cells upstream from, and in transcriptional control of, a first cistron, a second cistron downstream from the first cistron, under transcriptional control either of the first transcriptional promoter or under control of a second transcriptional promoter, optionally, a third cistron downstream from the second cistron, under transcriptional control either of the first transcriptional promoter or under control of the second transcriptional promoter, or under control of a third transcriptional promoter, and a transcriptional terminator following each of the first, second and third cistron, unless said first cistron or second cistron is followed by a second cistron or third cistron, respectively, which lacks its own transcriptional promoter; wherein each of the first, second and optionally third cistrons encode a combination of any two to three of the following:

1) tPA-gp120$_{MN}$;

2) gp160$_{IIIB}$/IRES/*REV*$_{IIIB}$;

3) gp160$_{IIIB}$;

4) *REV*$_{IIIB}$

5) *tat/REV*/gp160;

6) REV/gp160;

7) gp160$_{MN}$;

8) gp160 from [clinically relevant primary] a clinical HIV isolate [isolates];

9) nef, using the gene from [clinically relevant strains] a clinical HIV isolate;

10) *gag*$_{IIIB}$;

11) tPA-gp120$_{IIIB}$;

12) gp160 with structural mutations selected from the group consisting of [including] V3 loop substitutions from a clinical HIV isolate [clinically relevant strains of HIV]; several mutations on several constructs such as variable loop removal, Asn mutations to remove steric carbohydrate obstacles to structural, neutralizing antibody epitopes; and CD4 binding site knockout mutants;

13) gp41 with [provision of appropriate] a signal peptide leader sequence [sequences, as in the tPA signal peptide leader sequence];

14) *gag/REV*/gp160 [*gag*: similar to construct from #5 above, using the gene from clinically relevant strains];

15) *rev*: for gp160 and *gag* dicistronics;

16) a nucleotide sequence encoding B7 [coding sequences];

17) a nucleotide sequence encoding GM-CSF [sequences];
18) a nucleotide sequence encoding interleukin [Interleukin] sequences; and,
19) a nucleotide sequence encoding tumor [Tumor] associated antigens;
[20) Genes encoding antigens expressed by pathogens other than HIV, such as, but not limited to, influenza virus nucleoprotein, hemagglutinin, matrix, neuraminidase, and other antigenic proteins; herpes simplex virus genes; human papillomavirus genes; tuberculosis antigens; hepatitis A, B, or C virus antigens; and combinations of these and other antigens to form at least dicistronic constructs which may be combined with multiple other polycistronic constructs to provide a cocktail composition capable of raising immune responses against all of the represented pathogens or tumor antigens].

45(Amended). [The] A polynucleotide construct selected from the group consisting of V1Jns-(tat/rev SD), V1Jns-gp160$_{IIIB}$/IRES/rev $_{IIIB}$ (SD), V1Jns-gag-prt$_{IIIB}$ (SD), V1Jns-gag-prt$_{IIIB}$, V1Jns-tPA, V1Jns-tPA-gp120$_{MN}$, V1J-SIV$_{MAC251}$p28 gag, V1J-SIV$_{MAC251}$nef, and V1Jns-tat/rev/env.

New claim 46 was added as follows:

46(New). A polynucleotide which is non-replicating in eukaryotic cells *in vivo* and induces anti-HIV neutralizing antibody, HIV specific T-cell immune responses, or protective immune responses upon introduction into vertebrate tissue, including human tissue *in vivo*, wherein the polynucleotide comprises a gene encoding a gene product selected from the group consisting of HIV gag, HIV gag-protease, and HIV env, the gene containing a REV responsive element (RRE), the gene being operatively linked with a transcriptional promoter suitable for gene expression in a mammal, the gene being linked with an internal ribosome entry site (IRES), and the IRES being linked with a second gene, the second gene encoding a REV gene product.

New claim 47 was added as follows:

47(New). The polynucleotide of claim 39 wherein the HIV gene open reading frame is an HIV *gag* gene or portion thereof which encodes a *gag* immunogenic epitope.

New claim 48 was added as follows:

48(New). The polynucleotide of Claim 12 wherein the first cistron contains an HIV *gag* gene or portion thereof which encodes a *gag* immunogenic epitope, the second cistron encodes a cytokine, and the third cistron encodes a T-cell costimulatory element, wherein the first, second and third cistron may be presented in any combination.

New claim 49 was added as follows:

49(New). The polynucleotide of Claim 48 wherein the second cistron encodes an interleukin, an interferon, or GM-CSF, and the third cistron encodes a B7 protein.